

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A23C 9/127		A1	(11) International Publication Number: WO 94/21129 (43) International Publication Date: 29 September 1994 (29.09.94)
(21) International Application Number: PCT/DK94/00109 (22) International Filing Date: 18 March 1994 (18.03.94)		(81) Designated States: AU, CN, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 0312/93 19 March 1993 (19.03.93) DK		Published <i>With international search report.</i>	
(71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK).			
(72) Inventors; and (75) Inventors/Applicants (for US only): BUDOLFSEN, Gitte [DK/DK]; Lyøvej 3, 3.tv, DK-2000 Frederiksberg (DK). NIELSEN, Per, Munk [DK/DK]; Rytterstien 29A, DK-3400 Hillerød (DK).			
(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsvaerd (DK).			
(54) Title: METHOD FOR PRODUCTION OF AN ACIDIFIED EDIBLE GEL ON MILK BASIS, AND USE OF SUCH GEL			
(57) Abstract <p>The method for production of an acidified edible gel on milk basis comprises addition of transglutaminase to milk, followed by a heat treatment. Hereby a functionally and/or organoleptically satisfactory edible gel is obtained, which can be used as a yoghurt mousse or cheese.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

METHOD FOR PRODUCTION OF AN ACIDIFIED EDIBLE GEL ON MILK BASIS, AND USE OF SUCH GEL

Many efforts have been exercised in order to generate derived milk products of nutritional value and with improved functional and/or organoleptic properties, including acidified, edible gels on milk basis. A typical example of such products is desserts, especially yoghurt and curd. In order to prepare such desserts of satisfactory quality it is necessary either to add both emulsifying agents and stabilizing agents in rather large amounts or to conduct the production process as a slow fermentation.

10 It is the purpose of the invention to provide a method for production of an acidified edible gel on milk basis, which exhibits satisfactory functional and/or organoleptic properties differing from the prior art acidified edible gels on milk basis like yoghurt and curd, and in relation to which it is not necessary to add any emulsifying or stabilizing agents and in relation to which it is possible to conduct the 15 production process as a rapid pH reduction, and a use of such gel.

The method according to the invention for production of an acidified edible gel on milk basis is characterized by the fact that in a first step transglutaminase is added to milk or a milk like product, that in a second step the pH of the transglutaminase containing milk or milk like product is adjusted to 4.8 to 20 5.8, and that in a third step the pH-adjusted, transglutaminase containing milk or milk like product is exposed to a heat treatment.

In this specification with claims the term "milk or a milk like product" is to be understood as whole milk, skim milk, cream or a milk product with a fat content from 0% to 50% originating from any animal, preferably a cow, as such or 25 slightly modified, e.g. by addition of flavorants. Also, it is to be understood that the milk or milk like product can be produced by suspending skim milk powder and/or full fat milk powder in an aqueous medium. Furthermore it is to be understood that the protein content of the milk or milk like product, and thus also the protein content of the acidified edible gel, is of the order of magnitude 2.0 to 6.0% w/w.

30 Also, it goes without saying that the concentration of Ca^{++} is supposed to be of such value that Ca^{++} is able to activate the transglutaminase.

Transglutaminase can be added in a dosage measured in g of pure transglutaminase per g of the protein content of the milk product, or in a dosage based on the transglutaminase activity unit, indicated in P.D. Bishop et al., *Biochemistry*, 29, 1990, pp. 1861-1869.

Surprisingly it has been found that the gel which has a low protein content has a pleasant consistency and mouth feeling and exhibits satisfactory organoleptic properties. These organoleptic properties can be improved by addition of flavorants to the milk or milk like product. Also, it is surprising that no emulsifying or stabilizing agents need to be added in relation to the method according to the invention. The reason for this is not completely understood, but it may be assumed as a hypothesis that the transglutaminase crosslinks the proteins in the milk or milk like product, whereby a lattice or network is generated, which do not need either emulsifying or stabilizing agents due to its own inherent stability. Furthermore, it surprisingly has been found that the production of the acidified edible gel according to the invention can be carried out with a rapid pH reduction.

From Japanese unexamined patent application No. JP-A-3-160957 a gel on the basis of a transglutaminase modified milk protein is described. However, in the prior art no pH reduction is described, and also, the protein content of the milk or milk like product used as a starting material is around 10%, i.e. much higher than in relation to this invention.

In a preferred embodiment of the method according to the invention the transglutaminase is used in an amount of between 0.1 and 0.5% w/w, related to the amount of milk protein. In this manner an edible gel with satisfactory organoleptic characteristics can be obtained.

In a preferred embodiment of the method according to the invention the transglutaminase is of human, of bovine or of microbial origin. In this manner a transglutaminase with a satisfactory activity can be obtained.

In a preferred embodiment of the method according to the invention a heat treatment is carried out between the first and the second step, preferably at 30 a temperature between 60 - 100°C, and in a time range between 0.5 and 10 minutes.

In this manner an edible gel with improved organoleptic characteristics can be obtained.

In a preferred embodiment of the method according to the invention the milk or milk like product is whole milk, to which a flavorant has been added, preferably orange juice. In this manner an edible gel with improved organoleptic characteristics can be obtained.

5 In a preferred embodiment of the method according to the invention the heat treatment is carried out at a temperature between 60 and 140°C and a time range between 0.5 and 20 minutes, preferably at a temperature between 70 and 100°C and at a time range between 0.5 and 10 minutes. These intervals for temperature and time are optimal for the gelation of the transglutaminase treated
10 milk or milk like product.

Also, the invention comprises a use of the acidified edible gel on milk basis producible by means of the method according to the invention, as a yoghurt mousse, a cheese, or as a pickling liquid for meat. In regard to the use as a pickling liquid for meat it is to be noted 1) that the transglutaminase containing milk or milk
15 like product immediately before the heat treatment is injected into the meat or mixed intimately with the meat, and that the heat treatment is performed after the injection or the intimate mixing, and 2) that any kind of meat can be used in relation to this use, e.g. ham or fish meat. In relation to the use of the gel as a pickling liquid for meat it is to be noted that a gel cannot be injected into the meat; thus, in this case
20 the third step of the method for production of the gel will only be performed after the injection of the pH-adjusted, transglutaminase containing milk or milk like product into the meat.

The method according to the invention will be illustrated in the following example.

25 EXAMPLE 1

To 100 g milk (pH 6.68) is added 0.014 g of active human transglutaminase. 9.2 g of black currant juice and 11 g of orange juice is added, whereby pH of the total mixture changes to 5.20.

100 ml of the total mixture is subsequently treated in a microwave oven for 50 seconds with an effect of 520 watt, whereby an organoleptically acceptable gel is formed.

11 If a similar experiment without addition of transglutaminase is performed, the end product remains liquid.

EXAMPLE 2

To 100 g portions of reconstituted skim milk made with either 9%, 15% or 30% skim milk powder is added 0.014 g, 0.023 g and 0.047 g of active human transglutaminase (FXIIIa).

12 The portions are then incubated at 37°C for 45 minutes.

13 The portions are then acidified to pH 5.0 with HCl, Citric Acid and Glucone- delta- Lactone, respectively. The portions are then heated in a microwave oven for 50 seconds with an effect of 520 watt, whereby organoleptically acceptable gels are formed.

14 If a similar experiment without addition of transglutaminase is performed no gel is formed.

CLAIMS

1. Method for production of an acidified edible gel on milk basis, characterized by the fact that in a first step transglutaminase is added to milk or a milk like product, that in a second step the pH of the transglutaminase containing milk or milk like product is adjusted to 4.8 to 5.8, and that in a third step the pH-adjusted, transglutaminase containing milk or milk like product is exposed to a heat treatment.
2. Method according to Claim 1, characterized by the fact that the transglutaminase is used in an amount of between 0.1 and 0.5% w/w, related to the amount of milk protein.
3. Method according to Claim 1 or 2, characterized by the fact that the transglutaminase is of human, of bovine or of microbial origin.
4. Method according to Claims 1 - 3, characterized by the fact that between the first and the second step a heat treatment is carried out, preferably at a temperature between 60 - 100°C, and in a time range between 0.5 and 10 minutes.
5. Method according to Claims 1 - 4, characterized by the fact that the milk or milk like product is whole milk, to which a flavorant has been added, preferably orange juice.
6. Method according to Claims 1 - 5, characterized by the fact that the heat treatment is carried out at a temperature between 60 and 140°C and a time range between 0.5 and 20 minutes, preferably at a temperature between 70 and 100°C and at a time range between 0.5 and 10 minutes.

7. Use of the acidified edible gel on milk basis producible by means of the method according to Claims 1 - 6, as a yoghurt mousse, a cheese, or as a pickling liquid for meat.

1
INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 94/00109

A. CLASSIFICATION OF SUBJECT MATTER

IPC : A23C 9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : A23C, A23J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CLAIMS/US PATENTS, JAPIO, BIOSIS, AGRICOLA, CAB ABSTRACTS, FSTA, CA SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Service, File 50, CAB ABSTRACTS, Dialog accession no. 1258838, CAB accession no. 0D054-03006, Rao, D.S.: "Enzymatic modification of milk proteins during processing - a possible role for native milk proteinase.", Indian Dairyman 1991, 43 (11) p. 514-517 --	1-7
A	Chemical Abstracts, Volume 118, No 7, 15 February 1993 (15.02.93), (Columbus, Ohio, USA), Nonaka, M. et al., "Sodium caseinate and skim milk gels formed by incubation with microbial transglutaminase", page 713, THE ABSTRACT No 58314b, J. Food Sci. 1992, 57 (5), 1214-1241, (Eng.) --	1-7



Further documents are listed in the continuation of Box C.



See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

28 June 1994

Date of mailing of the international search report

06 -07- 1994

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. + 46 8 666 02 86Authorized Officer
INGA-KARIN PETERSSON
Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

2

International application No.

PCT/DK 94/00109

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chemical Abstracts, Volume 112, No 1, 1 January 1990 (01.01.90), (Columbus, Ohio, USA), page 618, THE ABSTRACT No 6095n, JP, A, 127471, (Ajinomoto Co., Inc. et al.) 30 January 1989 (30.01.89) --	1-7
A	Patent Abstracts of Japan, Vol 7, No 268, C-197, abstract of JP, A, 58-149645 (Ajinomoto K.K.), 6 Sept 1983 (06.09.83) --	1-7
A	Dialog Information Service, File 50, CAB ABSTRACTS, Dialog accession no. 1422994, CAB accession no. 0D055-03167, Ikura, K. et al.: "Use of transglutami- nase in quality-improvement and processing of food proteins.", Comments on Agricultural and Food Che- mistry 1992, 2 (6) p. 389-407	1-7
A	 --	1-7
A	Patent Abstracts of Japan, Vol 8, No 156, C-234, abstract of JP, A, 59-59151 (Ajinomoto K.K.), 4 April 1984 (04.04.84) -----	1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/94

International application No.

PCT/DK 94/00109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP-A- 127471	30/01/89	NONE	